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Draft Genome Sequences of Two Basal Members of the *Anaerolineae* Class of *Chloroflexi* from a Sulfidic Hot Spring

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ABSTRACT Here, we describe the first genome sequences of the *Anaerolineae* from a sulfidic environment, expanding the environmental distribution of sequenced *Anaerolineae*. These genomes represent basal *Anaerolineae* lineages, branching soon after the divergence of the sister class "*Candidatus* Thermofonsia," expanding our understanding of the metabolic evolution of this group.

Although members of the *Anaerolineae* class of the *Chloroflexi* phylum appear in diverse environmental 16S sequence data sets (e.g., carbonate tidal flats [1] and iron-rich hot springs [2]), the environmental distribution of previously sequenced *Anaerolineae* is primarily limited to groundwater and wastewater systems (e.g., references 3 and 4). Here, we present the first genomes of *Anaerolineae* from sulfidic environments, Nak19 and Nak57, expanding the genetic and environmental distribution of sequenced representatives of this clade.

The metagenome-assembled genomes (MAGs) were recovered from metagenomic sequencing of microbial communities from a sulfidic hot spring in Japan, as described previously (5, 6). In brief, samples were collected from microbial mats at Nakabusa Onsen in Nagano Prefecture, Japan, and DNA was extracted and sequenced via Illumina HiSeq. Sequences from four samples were coassembled with MegaHit version 1.1.2 (7) and genome bins constructed based on differential coverage using Metabat (8). Genome bins were assessed for completeness and contamination using CheckM (9) and uploaded to RAST for overall characterization (10).

MAG Nak19 totals 3.45 Mb recovered as 158 contigs, encoding 3,163 coding sequences and 44 tRNAs. The Nak19 genome has 51.2% GC content and was estimated by CheckM to be 90% complete, with 6.09% contamination. Nak57 is 3.77 Mb over 159 contigs, encoding 45 tRNAs and 3,391 coding sequences. Its GC content is 51.2%. CheckM estimates Nak57 to be 95.45% complete, with 4.85% contamination.

Neither Nak19 nor Nak57 recovered a 16S gene, but phylogenies based on RpoB and concatenated ribosomal proteins robustly place these MAGs as basal *Anaerolineae*, with Nak19 being the basal-most *Anaerolineae* and Nak57 being more closely related to cultured *Anaerolineae*, such as *Thermanaerothrix daxensis* (11) and *Ornatilinea apprima* (12).

Nak19 and Nak57 both encode aerobic respiration via an A-family heme-copper oxidoreductase (HCO) complex, a *bd* oxidase, and a *bc* complex. Nak57 also encodes an alternative complex III (ACIII), while Nak19 encodes NirS for nitrite reduction. Phylogenetic analysis of the respiration genes from Nak57 show gene relationships congruent with organismal relationships, supporting the vertical inheritance of aerobic respiration in the *Anaerolineae* following acquisition at the base of the class, consistent with trends in other *Chloroflexi* classes (6, 13). ACIII was not recovered in Nak19, but this may be a false-negative result due to incompleteness of the MAG; however, as the basal-most *Anaerolineae* member, it may also be that ACIII had not yet been acquired by the

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Anaerolineae when this clade diverged. ACIII may therefore have been acquired on the branch leading to Nak57 and the other *Anaerolineae*. The *Anaerolineae* have typically been described as obligately anaerobic fermenters (e.g., references 14 and 15), but genes for aerobic respiration appear to be widespread in this group (3, 4, 11). It remains uncertain whether these genes are used for aerobic respiration or only for O_2 detoxification in the *Anaerolineae* whose genomes are described here and elsewhere.

Consistent with other reports from sequenced *Chloroflexi*, Nak19 and Nak57 do not appear to encode outer membrane proteins, supporting interpretations of a monoderm membrane architecture as distinct from that of the diderm sister phylum *Armatimonadetes* (6, 16, 17).

Accession number(s). These whole-genome shotgun projects were deposited in DDBJ/EMBL/GenBank under the accession numbers QEXX00000000 (Nak19) and QEXW00000000 (Nak57).

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REFERENCES

1. Trembath-Reichert E, Ward LM, Slotznick SP, Bachtel SL, Kerans C, Grotzinger JP, Fischer WW. 2016. Gene sequencing-based analysis of microbial-mat morphotypes, Caicos Platform, British West Indies. J Sediment Res 86:629–636. https://doi.org/10.2110/jsr.2016.40.
2. Ward LM, Idei A, Terajima S, Kakegawa T, Fischer WW, McGlynn SE. 2017. Microbial diversity and iron oxidation at Okuoku-hachikurou Onsen, a Japanese hot spring analog of Precambrian iron formations. Geobiology 15:817–835. https://doi.org/10.1111/gbi.12266.
3. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Levilinea saccharolytica* KIBI-1, a member of the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01357-15. https://doi.org/10.1128/genomeA.01357-15.
4. Ward LM, Hemp J, Pace LA, Fischer WW. 2015. Draft genome sequence of *Leptolinea tardivitalis* YMTK-2, a mesophilic anaerobe from the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01356-15. https://doi.org/10.1128/genomeA.01356-15.
5. Ward LM. 2017. Microbial evolution and the rise of oxygen: the roles of contingency and context in shaping the biosphere through time. Ph.D. dissertation. California Institute of Technology, Pasadena, CA. https://doi.org/10.7907/Z9BZ642S.
6. Ward LM, Hemp J, Shih PM, McGlynn SE, Fischer WW. 2018. Evolution of phototrophy in the *Chloroflexi* phylum driven by horizontal gene transfer. Front Microbiol 9:260. https://doi.org/10.3389/fmicb.2018.00260.
7. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, Yamashita H, Lam T-W. 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.
8. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3:e1165. https://doi.org/10.7717/peerj.1165.
9. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25: 1043–1055. https://doi.org/10.1101/gr.186072.114.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
11. Pace LA, Hemp J, Ward LM, Fischer WW. 2015. Draft genome of *Thermanaerothrix daxensis* GNS-1, a thermophilic facultative anaerobe from the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01354-15. https://doi.org/10.1128/genomeA.01354-15.
12. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Ornatilinea apprima* P3M-1, an anaerobic member of the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01353-15. https://doi.org/10.1128/genomeA.01353-15.
13. Shih PM, Ward LM, Fischer WW. 2017. Evolution of the 3-hydroxypropionate bicycle and recent transfer of anoxygenic photosynthesis into the *Chloroflexi*. Proc Natl Acad Sci U S A 114:10749–10754. https://doi.org/10.1073/pnas.1710798114.
14. Yamada T, Sekiguchi Y. 2009. Cultivation of uncultured *Chloroflexi* subphyla: significance and ecophysiology of formerly uncultured *Chloroflexi* 'subphylum I' with natural and biotechnological relevance. Microbes Environ 24:205–216. https://doi.org/10.1264/jsme2.ME09151S.
15. Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, Kamagata Y. 2006. *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum *Chloroflexi*. Int J Syst and Evol Microbiol 56:1331–1340. https://doi.org/10.1099/ijs.0.64169-0.
16. Sutcliffe IC. 2011. Cell envelope architecture in the *Chloroflexi*: a shifting frontline in a phylogenetic turf war. Environ Microbiol 13:279–282. https://doi.org/10.1111/j.1462-2920.2010.02339.x.
17. Ward LM, McGlynn SE, Fischer WW. 2017. Draft genome sequences of a novel lineage of *Armatimonadetes* recovered from Japanese hot springs. Genome Announc 5:e00820-17. https://doi.org/10.1128/genomeA.00820-17.